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TITLE: Developing a PTEN-ERG Signature to Improve Molecular Risk Stratification in Prostate Cancer

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14. ABSTRACT

Based on previous work and our own preliminary data, it is clear that there exist distinctive molecular correlates of PTEN loss in the context of ETS-negative versus ETS-positive human prostate cancers and that these may drive prognosis following PTEN loss. Ultimately, elucidation of the molecular underpinnings of PTEN-ERG crosstalk holds promise for clarifying the mechanisms underlying the aggressive behavior seen in ERG-negative/PTEN-negative tumors, and may enable rational design of more effective therapeutic strategies and prognosticators for this subset of patients. We propose to test two hypotheses generated by our preliminary data: 1) PTEN loss in the absence of ETS gene expression is associated with an increased proliferative rate and a significantly increased risk of metastasis and death; and 2) ETS expression interacts with PTEN loss to modulate the transcriptional output from key oncogenic transcription factors such as MYC. Here we report on the initial studies performed for this award.

15. SUBJECT TERMS

Prostate cancer, PTEN, ERG, ETS, MYC, cell cycle, gene expression, RNA sequencing, Cap Analysis of Gene Expression (CAGE)

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1. INTRODUCTION:

PTEN and *ETS* family members such as ERG are among the most commonly altered genes in primary prostate cancer (PCa). PTEN and ERG molecular classification is inexpensive and widely accessible, however, our limited understanding of the interaction between these genes during PCa progression remains a major barrier to widespread implementation of these potential prognostic and predictive biomarkers. *PTEN* deletion is more common in ERG-positive than in ERG-negative PCa and almost always occurs after *ERG* rearrangement. In mouse models, PTEN loss synergizes with *ERG* expression to promote PCa progression. However, in recent human studies we have shown that PTEN loss is associated with lethal disease only among ERG-negative tumors (provisionally accepted at *JNCI*). Furthermore, though altered androgen signaling has been the focus of previously proposed mechanisms to explain the interaction of PTEN and ERG in murine models, our preliminary analyses in human tumors found key roles for cell cycle modulation and oncogenic transcription factor signaling. Clearly, additional work is required if we wish to exploit the power of these genomic classifiers for prognosis and therapy.

Based on previous work and our own preliminary data, it is clear that there exist distinctive molecular correlates of PTEN loss in the context of ETS-negative versus ETS-positive human PCa and that these may drive prognosis following PTEN loss. Ultimately, elucidation of the molecular underpinnings of PTEN-ERG crosstalk holds promise for clarifying the mechanisms underlying the aggressive behavior seen in ERG-negative/PTEN-negative tumors, and may enable rational design of more effective therapeutic strategies and prognosticators for this subset of patients. In the aims detailed below, we propose to test two hypotheses generated by our preliminary data: 1) PTEN loss in the absence of ETS gene expression is associated with an increased proliferative rate and a significantly increased risk of metastasis and death; and 2) ETS expression interacts with PTEN loss to modulate the transcriptional output from key oncogenic transcription factors such as MYC.

2. KEYWORDS:

KEYWORDS: Prostate cancer, PTEN, ERG, ETS, MYC, cell cycle, gene expression, RNA sequencing, Cap Analysis of Gene Expression (CAGE)

3. ACCOMPLISHMENTS:

What were the major goals of the project? (Only goals involving Dr. Lotan are listed below on this partnering PI award; please see Dr. Marchionni's report for his goals)

a. **Specific Aim 1:** Validate association of PTEN and ETS status with risk of lethal prostate cancer
    i. Assessing prostatectomy cohorts on multiple tissue microarrays (TMA) for PTEN, ETS, and cell proliferation rate (Months 1-36)
        1. Perform immunostaining for PTEN, ERG and Ki-67 and in situ hybridization on tissue microarrays (TMAs) from JHU and MSKCC cohorts; perform immunostaining for Ki-67 on HPFS/PHS cohort (Months 1-12; 100% completed)
        2. Score immunostaining and in situ hybridization (Months 13-24; 20% completed)

ii. Analysis of immunostaining and in situ hybridization data (Months 18-20; 20% completed)
iii. Co-author manuscript on association of PTEN/ETS status with cell cycle gene expression, proliferation rate and risk of metastasis and death in multiple validation cohorts (months 31-36; 0% completed)

b. **Specific Aim 3:** Discover and validate gene regulatory and expression signatures associated with PTEN loss on genetically homogeneous ERG-positive and ERG-negative backgrounds.
    i. Select 40 FFPE tumors from Johns Hopkins Surgical Pathology archives (20 ERG-positive and 20 ERG-negative, ETV1-negative). Within each group 10 have heterogeneous PTEN loss, 5 have homogeneous PTEN loss and 5 have intact PTEN by IHC (Months 1-12; 90% completed)
        1. Immunostaing 100 index tumors from Gleason 3+4=7 radical prostatectomies (Months 1-6; 100% competed)
        2. Score staining and select cases (Months 4-8; 100% completed)
        3. Punch blocks and prepare RNA for CAGE (months 8-12; 80% completed)

What was accomplished under these goals?

1) Major activities during this reporting period include HRPO and IRB approval of all studies and performing immunostaining and in situ hybridization on the JHU and MSKCC tissue microarray (TMA) cohorts. We have performed and scored PTEN and ERG immunostaining on the JHU and MSKCC TMA cohorts, in addition to ETV1, ETV4 and ETV5 in situ hybridization on the JHU cohorts. Ki-67 immunostaining has been performed as proposed on both cohorts and automated scoring is pending. We also began to analyze the PTEN/ERG/ETS data for association with metastasis and death from prostate cancer in the JHU and MSKCC cohorts. In addition, we have gathered a cohort of ~50 prostate tumors (Gleason 7) at radical prostatectomy where tissues are less than one year of age and we have performed PTEN and ERG immunostaining for future use in CAGE assays. Since these are formalin-fixed paraffin embedded tissues, separately, Dr. Marchionni has collaborated to collect fresh frozen tissues on an additional ~20 cases and is assessing different CAGE protocols optimized for minimal RNA input quantities before proceeding with CAGE.

2) Specific objectives during this reporting period were to ascertain PTEN/ERG/ETS status of tumors in the JHU and MSKCC cohort, correlate these data with gene expression data from the same cohort to confirm ETS status and enable full gene expression analyses of these cohorts to proceed in the next year of the award. In addition, we aimed to reproduce data previous published using the HPFS/PHS cohorts to demonstrate that PTEN loss is associated with a worse outcome when it occurs on an ERG/ETS-negative background in the MSKCC and JHU cohorts.

3) Significant results or key outcomes included HRPO approval and the finding that PTEN loss is associated with lethal prostate cancer in a multivariable model using the MSKCC cohort, particularly when it occurs in ERG-negative prostate cancer, as we showed

previously (Ahearn et al, *JNCI*, 2015). The analysis is presented in Table 1 below. A similar analysis for the JHU cohort is pending.

Table 1. Cox Analysis of Hazard Rate (HR) of Lethal Prostate Cancer in MSKCC cohort

Lethal Prostate Cancer		Univariable Analysis		Multivariable Analysis[a]	
	N(Case/Control)	HR (95% CI)	p value	HR (95% CI)	p value
PTEN_ERG					
PTEN Intact/ERG Negative	35/328	Reference		Reference	
PTEN Intact/ERG Positive	10/200	0.47 (0.23-0.96)	0.04	0.41 (0.16-1.07)	0.1
PTEN Loss/ERG Negative	27/56	3.76 (2.27-6.21)	<0.0001	2.49 (1.40-4.43)	0.002
PTEN Loss/ERG Positive	20/100	1.84 (1.06-3.18)	0.03	1.15 (0.59-2.23)	0.7
PTEN Intact					
ERG Negative	35/328	Reference		Reference	
ERG Positive	10/200	0.47 (0.24-0.96)	0.04	0.42 (0.16-1.11)	0.1
PTEN Loss					
ERG Negative	27/56	Reference		Reference	
ERG Positive	20/100	0.49 (0.28-0.87)	0.02	0.48 (0.23-1.04)	0.1
PTEN Heterogeneous Loss					
ERG Negative	7/28	Reference		Reference	
ERG Positive	6/55	0.44 (0.15-1.32)	0.1	0.22 (0.02-2.13)	0.2
PTEN Homogeneous Loss					
ERG Negative	20/28	Reference		Reference	
ERG Positive	14/45	0.59 (0.30-1.17)	0.1	0.57 (0.24-1.35)	0.2

[a] Adjusted for age at radical prostatectomy, race, gleason score and stage.

What opportunities for training and professional development has the project provided?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we will focus on finishing the ki-67 immunohistochemistry scoring, which will be done using automated digital image analysis. In addition, a major goal of the next reporting period is working with Dr. Marchionni to begin to analyze gene expression data for each TMA set and correlation to PTEN/ERG status. Dr. Marchionni has begun this work and we will begin to plan a manuscript to accomplish Milestone #1. Finally, preparation of RNA for CAGE analysis will begin in earnest and we will decide based on preliminary data whether to proceed with fresh or frozen tissues for this aim.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

We have successfully reproduced data indicating that PTEN loss is more significant when it occurs on an ERG/ETS-negative background, corroborating the results of our previous HPFS/PHS study in an independent cohort.

We have successfully applied highly validated IHC and in situ hybridization assays to determine PTEN and ETS status in 2 additional cohorts (MSKCC and JHU) with accompanying gene expression data for analysis

No molecular signatures of PTEN loss in prostate cancer have been developed to date, thus this project will add significantly to prostate cancer research by further refinement and validation of this prognostic biomarker as we develop expression signatures in the next reporting periods.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

We have had a slight delay in the Ki-67 image analysis as we seek the best platform for this digital scoring. We performed scoring on a test TMA using the Aperio platform and had difficulty determining the denominator of total cancer cells within each field. We are now using a darker counter stain with hematoxylin to address this issue.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations

Nothing to report

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products
Database of PTEN/ERG/ETS status in HPFS/PHS cohort, JHU Natural History cohort and MSKCC cohort. We will make this available to other researcher upon publication.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Tamara Lotan*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*Tlotan1*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Lotan supervised IHC and ISH data collection and interpretation.*
Funding Support:	*NCI/NIH, Prostate Cancer Foundation, CDMRP-PCRP*

Name:	*Alba Torres*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Torres performed IHC and ISH data collection and interpretation.*
Funding Support:	*CDMRP-PCRP*

Name:	*Kaushal Asrani*
Project Role:	*Postdoctoral fellow*

Researcher Identifier (e.g. ORCID ID):	*Kasrani1*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Asrani supervised data collection and interpretation.*
Funding Support:	*NCI/NIH, CDMRP-PCRP*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Lotan:
No longer supported by W81XWH-13-1-0271—this award is completed.
No longer supported by W81XWH-12-PCRP-TIA—this award is completed.
W81XWH-17-1-0425 —this award is now active and moved from pending to current.
W81XWH-17-1-0286 —this award is now active and moved from pending to current

What other organizations were involved as partners?

Organization Name: Memorial Sloan Kettering Cancer Center

Location of Organization: New York, New York, USA

Partner's contribution to the project

Collaboration : Dr. Anu Gopalan is a pathologist who created the MSKCC TMAs described above and she has participated in scoring and data analysis of these materials after providing them to us (<1 person/month effort).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Dr. Marchionni (Partnering PI) is submitting an independent report detailing the work he is overseeing on this project separately.

9. APPENDICES: None